(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/74716 A2

(51) International Patent Classification[7]: A61K 39/385, A61P 37/08

(21) International Application Number: PCT/EP00/05164

(22) International Filing Date: 6 June 2000 (06.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9913327.4 8 June 1999 (08.06.1999) GB

(71) Applicant *(for all designated States except US)*: **SMITHKLINE BEECHAM BIOLOGICALS S.A.** [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **PRIEELS, Jean-Paul** [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).

(74) Agent: **DALTON, Marcus, Jonathan, William**; SmithKline Beecham, Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/74716 A2

(54) Title: VACCINE

(57) Abstract: The present invention provides a composition for the prophylaxis or treatment of allergy. The composition comprises a plurality of allergy peptides linked together by an inert carrier; characterised in that said allergy peptides are derived from IgE or IgE receptor, and that the inert carrier does not contain a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation, and in a preferred embodiment the carrier does not provide bystander T-cell cytokine help in the induction of the anti-peptide immune response. Also provided are methods of their manufacture and their use in medicine. There is also provided a method of treatment of allergy comprising priming the immune system with an IgE peptide/protein conjugate, followed by boosting the immune response with a composition of the present invention.

Vaccine

The present invention provides a composition for the prophylaxis or treatment of allergy. The composition comprises a plurality of allergy peptides linked together by an inert carrier; characterised in that said allergy peptides are derived from IgE or IgE receptor, and that the inert carrier does not contain a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation, and in a preferred embodiment the carrier does not provide bystander T-cell cytokine help in the induction of the anti-peptide immune response. Also provided are methods of their manufacture and their use in medicine. The compositions of the present invention are especially useful in the context of regular booster vaccine doses, after priming dose of vaccine comprising the IgE peptide conjugated to a protein carrier. Accordingly, there is provided a method of treatment of allergy comprising priming the immune system with an IgE peptide/protein conjugate, followed by boosting the immune response with a composition of the present invention.

Immunogens comprising short peptides are becoming increasingly common in the field of vaccine prophylaxis or therapy. As such, in many disease states it is often possible, and desirable, to design vaccines comprising a short peptide rather than the large protein. The peptides which are used may be the full length native immunogen, for example human peptidic hormones, or may be fragments of a larger antigen derived from a given pathogen, or from a large self-protein. For example, short peptides of IgE may be used for prophylaxis of allergy, whereas the use of IgE itself as the immunogen may induce anaphylactic shock.

It has previously been thought that amongst the problems associated with the peptide approach to vaccination, is the fact that peptides *per se* are poor immunogens. Generally the sequence of the peptides are chosen such that they include a B-cell epitope to provide a target for the generation of anti-peptide antibody responses, but because of their limited size rarely encompass sufficient T-cell epitopes in order to

provide the necessary cytokine help in the induction of strong immune responses following priming and boosting applications of the vaccine.

Strategies to overcome this problem of immunogenicity include the linking of the peptide to large highly immunogenic protein carriers. The carrier proteins contain a large number of peptidic T-cell epitopes which are capable of being loaded into MHC molecules, thereby providing bystander T-cell help, and/or alternatively the use of strong adjuvants in the vaccine formulation.

Examples of these highly immunogenic carriers which are currently commonly used for the production of peptide immunogens include the Diptheria and Tetanus toxoids (DT and TT respectively), Keyhole Limpet Haemocyanin (KLH), and the purified protein derivative of Tuberculin (PPD). A number of problems are associated with each of these commonly used carriers, including both problems in production of GMP constructs and also in immunological characteristics of the constructs.

Despite the common use of these carriers and their success in the induction of high anti-peptide antibody responses they are associated with several drawbacks. For example, it is known that antigen specific immune responses may be suppressed by the presence of pre-existing antibodies directed against the carrier, for example Tetanus toxin (Di John *et al*; Lancet, December 16, 1989). In the population at large, a very high percentage of people will have pre-existing immunity to both DT and TT as people are routinely vaccinated with these antigens. In the UK for example 95% of children receive the DTP vaccine comprising both DT and TT. Other authors have described the problem of epitope suppression to peptide vaccines in animal models (Sad *et al*, Immunology, 1991; 74:223-227; Schutze *et al*, J. Immunol. 135: 4, 1985; 2319-2322).

In addition, for vaccines which require regular boosting, the use of highly immunogenic carriers such as TT and DT are likely to suppress the peptide antibody response after several injections. These multiple vaccinations may also be

accompanied by undesireable reactions such as delayed type hyperresponsive reactions (DTH) to the carrier.

One of the fields of research where a great deal of effort has been made in designing peptide based vaccines in the prophylaxis and therapy of allergic responses. In a response to allergens, the symptoms of an allergic response are brought about by the release of mediators (such as histamine) from immune cells into the surrounding tissues and vascular structures. Histamine is normally stored in mast cells or basophils, until such time as the release is triggered by interaction with allergen specific IgE.

The role of IgE in the mediation of allergic responses, such as asthma, food allergies, type-I hypersensitivity and sinus inflammation, is well known. On encountering an antigen, such as pollen or dust mite allergens, B cells commence the synthesis of allergen specific IgE. The allergen specific IgE then binds to its FcεRI receptor (the high affinity receptor) on basophils and mast cells. Any subsequent encounter with allergen leads to the triggering of histamine release from the mast cells or basophils, and cross-linking of neighbouring IgE/ FcεRI complexes (EP 0 477 231 B1).

IgE, like all immunoglobulins, comprises two heavy and two light chains. The ε heavy chain consists of five domains: one variable domain (VH) and four constant domains (Cε1 to Cε4). The molecular weight of IgE is about 190,000 Da, the heavy chain being 550 amino acids in length. For a review of IgE and its interaction with the high and low affinity IgE receptors (FcεRI and FcεRII) see Sutton and Gould (Nature, 1993, Vol. 386, 421-428).

A number of passive or active immunotherapeutic and immunoprophylactic approaches which interfere with this IgE-mediated histamine release mechanism have been investigated. These approaches range from the prevention of allergen/IgE complexes from binding to the FcεRI or FcεRII receptors on mast cells with passively administered antibodies, or competitive binding of IgE to the receptors by IgE derived

peptides; to the use of specific IgE peptides for active immunisation to stimulate histamine release inhibiting immune responses.

Therefore, in order to be safe and effective, the passively administered or vaccine induced antibodies must bind in a region which is capable of interfering with the histamine triggering pathway, without being anaphylactic *per se*. Furthermore, in order to maintain high levels of prophylactically and therapeutically effective anti-peptide antibodies in vaccinated individuals, it is envisaged that the individuals will receive regular and frequent booster vaccinations.

The highly immunogenic carriers used previously for allergy peptide vaccines, whilst they may induce high levels of anti-peptide antibody responses in naïve individuals on primary immunisation, therefore have many disadvantages in the context of a population with pre-existing immunity to the carrier and also in the context of regular boosting. Accordingly, there is a need for the development of allergy peptide based compositions for regular booster vaccinations which do not contain such large protein carrier molecules.

EP 0 477 231 B1 describes one approach to allergy immunotherapy. Peptides derived from the Cε4 domain of IgE (residues 497-506, also known as the Stanworth decapeptide), conjugated to Keyhole Limpet Haemocyanin (KLH). A number of carriers for the IgE peptides are suggested including KLH, Tetanus toxoid, Diptheria toxoid, albumins, haemocyanins such as Keyhole Limpet Haemocyanin (KLH), polymers of amino acids, and preferably purified protein derivative of tuberculin (PPD).

KLH is known as potent immunogen and has already been used as a carrier for IgE peptides in human clinical trials. However, some adverse reactions (DTH-like reactions or IgE sensitisation) as well as antibody responses against KLH which could compete with the anti-IgE peptide antibody have been observed. Indeed, it has been reported that antisera induced by such immunogens frequently contain more

anti-KLH antibodies than to the target peptide (Stanworth and Burt, 1986, *Mol. Immunol.*, 23, 1231-1235).

WO 95/26365 further continues investigation of the Cε4 (497-506) peptide and describes immunogens comprising additional universal T-helper epitopes. The immunogens of WO 95/26365 are in the general formula:

$(A)_n$-$(Th)_m$-$(B)_o$-$(Stan)_p$

where A is an amino acid; Th is a T-helper epitope; B is an amino acid; and Stan is the Stanworth decapeptide. n=1 to 10; m=1 to 4; o=0 to 10; and p=1 to 3.

WO 98/24808 describes oligopeptides derived from Cε3, which interact with the high or low affinity receptors of IgE. These oligopeptides are expressed as fusion proteins together with the expression partner Glutathione-S-transferase (GST). WO 97/31948 describes IgE peptide immunogens conjugated to a protein carrier, the carriers described are TT, DT, KLH and PPD.

Peptide based vaccines in the absence of protein carriers have been described for other disease states; including peptides derived from Hepatitis B virus and *Streptococcus mutans* have been conjugated *via* the immunostimulatory polysaccharide, mannan (Lett *et al.*, Infection and Immunity, 1994, 785-792; Okawa *et al.*, Journal of Immunological Methods, 1992, 149, 127-131; Steward *et al.*, Vaccine, 1993, 11 (14): 1405-14). Also Hepatitis peptides have been linked via liposomes, polylysine and diaminoalkane carriers (*Neurath et al., 1984, Journal of General Virology, 65 (Pt 5):1009-14; Neurath et al., 1984, Virus Research; 1(4):321-31).* Tandem repeats of the peptide hormone GnRH have recently been described conjugated to branched polylysine structures or by lipo-thioester, for the immunocastration of pigs (Beekman *et al.*, 1999, Vaccine, 17, 2043-2050).

The present invention results from the surprising observation that peptide carriers comprising T-cell helper epitopes are not required for the boosting of anti-peptide responses. Thus, booster vaccines may comprise multimeric constructs of allergy peptides, which constructs do not contain additional highly immunogenic carrier

proteins. Without the need for an immunogenic carrier, such as Tetanus toxoid, in the booster vaccines; the problems of epitope suppression and pre-existing immunity to the carrier protein do not apply.

Accordingly there is provided a composition comprising a plurality of allergy peptides linked by an inert carrier; characterised in that the allergy peptides are derived from IgE or IgE receptor, and that the inert carrier does not contain a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation, and in a preferred embodiment the carrier does not provide bystander T-cell cytokine help in the induction of the anti-peptide immune response. Alternatively said constructs may consist of a complex of peptides, without any separate carrier. Such constructs described above are especially useful in the context of an allergy booster vaccine, wherein the booster vaccine is administered to individuals who have previously been primed with a primary vaccine which may comprise the same allergy peptide conjugated to a highly immunogenic carrier protein.

The term allergy peptide within the meaning of the present invention is a polymer of amino acids joined by peptide bonds, which peptides are derived from proteins associated with the generation of an allergic response, such that said peptides are capable of inducing an immune response which is capable of inhibiting an immune response which inhibits the allergy response. Thus, said peptides may contain a sequence of amino acids derived from the sequence of IgE or an IgE receptor (FcεRI or FcεRII), preferably said peptides comprising between 2-50 amino acids in length, and more preferably between 2-30 amino acids in length, and most preferably between 5-25 amino acids in length. The peptides of the present invention may be either lipidated or non-lipidated.

Preferably the peptides are derived from mammalian IgE, such that the compositions of the present invention are capable of stimulating a non-anaphylactic anti-IgE antibody response in a vaccinated individual. For example, the stanworth decapeptides as described in EP 0 477 231 B1, for example KTKGSGFFVF or mimetopes thereof.

Other IgE peptides are described in WO 97/31948 and WO 96/14333, and are useful in immunogens of the present invention.

IgE peptides which may advantageously be used in compositions of the present invention are described in table 1:

Table 1,

Sequence	SEQ ID NO.
EDGQVMDVD	1
STTQEGEL	2
SQKHWLSDRT	3
GHTFEDSTKKCADSNPRGV	4
CADSNPRGV	5
CLEDGQVMDVDLL	6
CSTTQEGELA	7
CSQKHWLSDRT	8
RASGKPVNHSTRKEEKQRNGTL	9
GTRDWIEGE	10
PHLPRALMRSTTKTSGPRA	11
PEWPGSRDKRT	12
EQKDE	13
CRASGKPVNHSTRKEEKQRNGLL	14
CGTRDWIEGLL	15
CHPHLPRALMLL	16
APEWPGSRDKRTC	17
KTKGSGFFVF	18

Alternatively, the allergy peptide may be derived from an IgE receptor. Said IgE receptor peptide may be derived from FcεRI or FcεRII, which generates anti-allergy immune responses. Particularly preferred peptides are SEQ ID NOs. 1, 6, and 17.

The present invention includes the native peptides themselves, and also any mimetope thereof. The meaning of mimetope is defined as a peptide sequence which is sufficiently similar to the native peptide (sequentially or structurally), which is capable of being recognised by antibodies which recognise the native peptide; or are capable of raising antibodies, when coupled to a suitable carrier, which antibodies are capable of recognising the native peptide or the native protein from which the peptides were identified.

Peptide mimetopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides may be modified for the purposes of ease of conjugation to the inert carrier. Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the IgE peptides of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native IgE peptide. This approach may have significant advantages by allowing the possibility of identifying a peptide with enhanced immunogenic properties (such as higher affinity binding characteristics to the IgE receptors or anti-IgE antibodies), or may overcome any potential self-antigen tolerance problems which may be associated with the use of the native peptide sequence. Accordingly, in one aspect of the invention there is provided mimetopes of the IgE peptides noted above.

The allergy peptides are conjugated to inert carriers to form compositions of the present invention. The term "inert carrier" within the meaning of the present invention is that inert carrier does not contain a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation, and in a preferred embodiment the carrier does not provide bystander T-cell cytokine help in the induction of the anti-peptide immune response. The carriers for use in the compositions of the present invention are chosen to allow multimeric presentation of the allergy peptides. It is clear to one skilled in the art that multimeric

presentation of peptides can be achieved in many ways and that any carrier which has multiple available conjugation sites can be considered. The carrier can be a linear or branched homo- or hetero-polymer, or can be a particulate structure composed of self-associating monomers such as lipids, or polymers.

Specific inert carriers to which allergy peptides could be linked to form compositions of the present invention include and is not restricted to the following: polysaccharides, either linear or branched, lipo-polysaccharides, poly-aminoacid homopolymers and block copolymers as well as blockcopolymers of aminoacids and polymers such as polyethyleneglycol or polycaprolactone, liposomes and other vesicular structures such as niosomes, novasomes, and also particulate structures such as nanoparticles, supramolecular biovectors or microspheres composed of polymers. Also, the allergy peptides can themselves be polymerised by methods known in the art, without attachment to a polymeric carrier. Peptides may be associated with liposomes by conjugation to phospholipids which are then formulated into the liposome bylayer, usingtechniques well known in the art (Friede et al., *Vaccine* 1994 Jul;12(9):791-7).

Particularly preferred carriers are polysaccharides derived from bacteria, including those derived from *Streptococcus pneumoniae*, for example PS6B (Zon, G. *et al.*, Infection and Immunity, 1982, 37, 89-103). Other preferred polysaccharides are *Streptococcus pneumoniae* 1,3,5, 7F, 9V, 14, 18C, 19F, 23F, *Haemophilus influenzae b*, *Neisseria meningitidis* A,C.

Vaccines of the present invention are preferably used for the prophylaxis or therapy of allergy.

Vaccines of the present invention comprise an allergy peptide composition as described above, an adjuvant, and a pharmaceutically acceptable excipient. Suitable adjuvants are well known in the art (Vaccine Design – The Subunit and Adjuvant Approach, 1995, Pharmaceutical Biotechnology, Volume 6, Eds. Powell, M.F., and Newman, M.J., Plenum Press, New York and London, ISBN 0-306-44867-X).

Preferred adjuvants for use with immunogens of the present invention include: aluminium or calcium salts (hydroxide or phosphate), oil in water emulsions (WO 95/17210, EP 0 399 843), or particulate carriers such as liposomes (WO 96/33739). Immunologically active saponin fractions (*e.g.* Quil A) having adjuvant activity derived from the bark of the South American tree Quillaja Saponaria Molina are particularly preferred. Derivatives of Quil A, for example QS21 (an HPLC purified fraction derivative of Quil A), and the method of its production is disclosed in US Patent No. 5,057,540. Amongst QS21 (known as QA21) other fractions such as QA17 are also disclosed. 3 De-O-acylated monophosphoryl lipid A is a well known adjuvant manufactured by Ribi Immunochem, Montana. It can be prepared by the methods taught in GB 2122204B. A preferred form of 3 De-O-acylated monophosphoryl lipid A is in the form of an emulsion having a small particle size less than 0.2μm in diameter (EP 0 689 454 B1).

Adjuvants also include, but are not limited to, muramyl dipeptide and saponins such as Quil A, bacterial lipopolysaccharides such as 3D-MPL (3-O-deacylated monophosphoryl lipid A), or TDM. As a further exemplary alternative, the protein can be encapsulated within microparticles such as liposomes, or in non-particulate suspensions or aqueous solutions of polyoxyethylene ether of general formula (I)

$HO(CH_2CH_2O)_n$-A-R

wherein, n is 1-50, A is a bond or -C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl (WO 99/52549).

Particularly preferred adjuvants are combinations of 3D-MPL and QS21 (EP 0 671 948 B1), oil in water emulsions comprising 3D-MPL and QS21 (WO 95/17210, PCT/EP98/05714), 3D-MPL formulated with other carriers (EP 0 689 454 B1), or QS21 formulated in cholesterol containing liposomes (WO 96/33739), or immunostimulatory oligonucleotides (WO 96/02555).

Examples of suitable pharmaceutically acceptable excipients include water, phosphate buffered saline, isotonic buffer solutions. The vaccines of the present invention may also be administered via the oral route. In such cases the pharmaceutically acceptible excipient may also include alkaline buffers, or digestible capsules.

The formulations of the present invention maybe used for both prophylactic and therapeutic purposes. Accordingly, the present invention provides for a method of treating allergy. In a further aspect of the present invention there is provided an allergy vaccine as herein described for use as a medicament. Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978.

In a further aspect of the present invention there is provided a method of producing an allergy vaccine comprising manufacturing an allergy peptide immunogen as herein described and formulating said immunogen with an adjuvant.

Specifically, there is provided the use of an allergy vaccine comprising one or a plurality of allergy peptides linked to an inert carrier, characterised in that said allergy peptides are derived from IgE or IgE receptor, and that said inert carrier does not contain a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation; to boost an anti-allergy immune response in an individual susceptible to an allergic response, characterised in that the immune system of said individual has previously been primed with a composition comprising the allergy peptide and a carrier comprising a T-helper epitope.

The vaccine preparation of the present invention may be used to protect or treat a mammal susceptible to, or suffering from, allergies, by means of administering said vaccine via systemic or mucosal route. These administrations may include injection *via* the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or *via* mucosal administration to the oral/alimentary, respiratory, genitourinary tracts.

It is expected that the boosting doses will be adequately spaced, or preferably given yearly or at such times where the levels of circulating antibody fall below a desired level. In an alternative related aspect of the present invention the vaccines may be

administered for both priming and boosting doses, when formulated with strong adjuvants such as 3D-MPL or QS21.

Additionally, there is provided a method of boosting an immune response, by administering a composition of claim 1 to an individual susceptible to an allergic response, characterised in that the immune system of said individual has previously been primed with a composition comprising the allergy peptide and a carrier molecule comprising a T-helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 μg of protein, preferably 1-500 μg, preferably 1-100 μg, of which 1 to 50μg is the most preferable range. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisations adequately spaced.

In a preferred embodiment of the present invention the vaccines of the present invention will be used to boost the anti-allergy immune response in individuals that have previously received a priming dose of a vaccine comprising an anti-allergy peptide conjugated to a T-helper epitope bearing carrier. In this booster vaccination regime the individuals will have previously been vaccinated with an allergy peptide conjugated to a carrier. The types of carriers comprising T-helper epitopes which are suitable to be used in the priming immunogens will be readily known to the man skilled in the art. A non-exhaustive list of carriers which may be used in the present invention include: Keyhole limpet Haemocyanin (KLH), serum albumins such as bovine serum albumin (BSA), inactivated bacterial toxins such as tetanus or diptheria toxins (TT and DT), of recombinant fragments thereof (for example, Domain 1 of Fragment C of TT, or the translocation domain of DT), or the purified protein

derivative of tuberculin (PPD). Alternatively the peptides may be directly conjugated to liposome carriers, which may additionally comprise immnuogens capable of providing T-cell help. In this embodiment of the invention a preferred carrier is Protein D from *Haemophilus influenzae* (EP 0 594 610 B1). Protein D is an IgD–binding protein from *Haemophilus influenzae* and has been patented by Forsgren (WO 91/18926, granted EP 0 594 610 B1). In some circumstances, for example in recombinant immunogen expression systems it may be desirable to use fragments of protein D, for example Protein D 1/3rd (comprising the N-terminus of protein D (GB 9717953.5)). Chemical conjugation methods for conjugating the IgE peptides to the carrier are also well known in the art and include gluteraldehyde, and the common commercially available heterobifunctional linkers such as CDAP, GMBS and SPDP (using manufacturers instructions).

Further, there is provided a method of inducing and maintaining an anti-allergy effective immune response comprising: (a) administering to an individual a composition comprising an allergy peptide conjugated to a T-cell epitope containing carrier; and (b) a subsequent administration to said individual of a composition comprising said allergy peptide in the absence of a T-cell epitope containing carrier

Moreover there is provided, use of an allergy peptide linked to an inert carrier; characterised in that said allergy peptide is derived from IgE or IgE receptor, and that said inert carrier does not contain a T-cell helper epitope in the manufacture of a medicament for the prophylaxis or treatment of allergy.

The invention is illustrated by, but not limited to, the following examples.

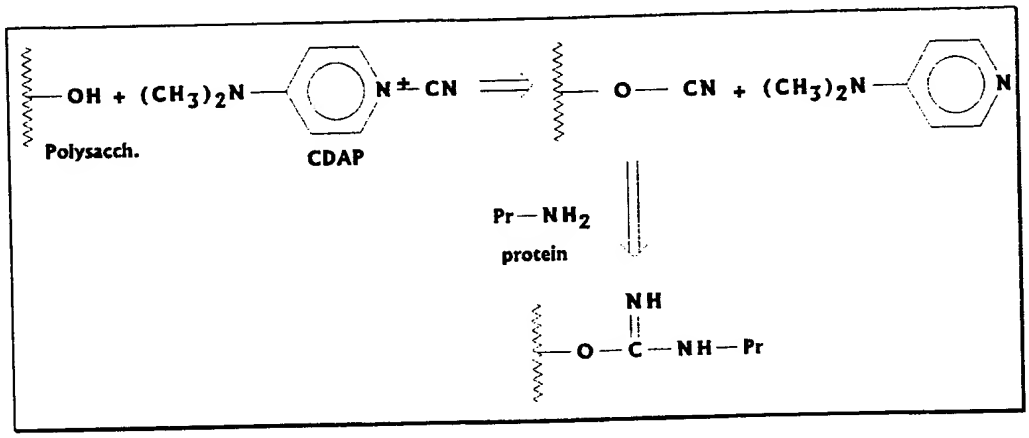
Example 1,

1.1 Synthesis of conjugates

Allergy peptides may be synthesised using known techniques, and may correspond to the stanworth decapeptide (KTKGSGFFVF) or other IgE peptides (see table 1). A

plurality of these allergy peptides may be linked to form a composition within the meaning of the present invention, via a polysaccharide "backbone". In this form of conjugate the cyanylating reagent 1-cyano-4-dimethylamino-pyridinium tetrafluoroborate (CDAP) is used for the synthesis of polysaccharide-peptide conjugates. CDAP is a water soluble reagent. The cyanilation reaction can be performed under relatively mild conditions and avoid hydrolysis of alkaline sensitive polysaccharides. It was found to activate a large range of polysaccharides as *Streptococcus pneumoniae* 1,3,5, 6B, 7F, 9V, 14, 18C, 19F, 23F, *Haemophilus influenzae b*, *Neisseria meningitidis* A,C. This synthesis allows direct coupling to a carrier protein or peptide.

The polysaccharide is solubilized in water or a saline solution. CDAP is dissolved in acetonitrile and added immediately to the polysaccharide solution. The CDAP reacts with the hydroxyl groups of the polysaccharide to form cyanate ester. After the activation step, the peptides are added. Amino groups of the peptides react with the activated polysaccharide to form an iso-urea linking. pH is under control during all the reaction.



After the coupling reaction, a large excess of glycine is then added to quench residual activated functions. The product is then passed through a gel permeation to remove unreacted carrier protein and residual reagents.

A dry powder of native polysaccharide from *Streptococcus pneumonae* (PS6B) prepared according to Zon, G. *et al.*, Infection and Immunity, 1982, 37, 89-103, and dissolved for 1 hour in water for injection at a concentration of 5 mg/ml. The solution is thermoregulated at 25°C and pH adjusted to a value of 6. CDAP, 0.75 per mg of polysaccharide (a preparation to 100 mg/mg CH_3CN) is added. After 1.5 minutes without pH regulation, triethylamine 0.2 M is added to obtain a pH of 9. 2 minutes later, a solution of peptide (PEP1 - concentrated at 3 mg/ml EPI) is added to the activated polysaccharide and pH maintained at a value of 9 by triethylamine during 1 hour. The initial peptide PEP1 / polysaccharide is 0.30 (w/w). 7.5 mg of glycine per mg of polysaccharide (a 2 M glycine solution, pH 9) is added for the quenching step during 30 minutes. The excess of residual component are removed by dialysis against NaCl 150 mM. The mixture is then purified onto a permeation gel sephacryl 400HR. The peptide-PS construct elutes first near the void volume and is pooled. A resorcinol and microBCA tests are achieved to determine peptide/PS ratio and peptide recovery.

The compositions thus produced may be formulated with an adjuvant to form a vaccine, examples of which include aluminium hydroxide (available from Superfos) or an oil in water emulsion. A preferred oil in water emulsion further comprises QS21 and or 3D-MPL, formulations of one such emulsion is described below:

1.2 Production of Oil in Water (o/w) emulsion adjuvants

The oil in water emulsion adjuvant formulations used in the subsequent examples were each made comprising the following oil in water emulsion component: 5% Squalene, 5% α-tocopherol, 2.0% polyoxyethylene sorbitan monooleate (TWEEN 80).

The emulsion was prepared as follows as a 2 fold concentrate. All examples used in the immunological experiments are diluted with the addition of extra components and diluents to give either a 1x concentration (equating to a squalene:QS21 ratio (w/w) of 240:1) or further dilutions thereof.

Briefly, the TWEEN 80 is dissolved in phosphate buffered saline (PBS) to give a 2% solution in the PBS. To provide 100 ml of a two fold concentrate emulsion, 5ml of DL alpha tocopherol and 5ml of squalene are vortexed to mix thoroughly. 95ml of PBS/TWEEN solution is added to the oil and mixed thoroughly. The resulting emulsion is then passed through a syringe needle and finally microfluidised by using an M110S Microfluidics machine. The resulting oil droplets have a size of approximately 145-180 nm (expressed as z av. measured by PCS) and is termed SB62.

The other adjuvant/vaccine components (QS21, 3D-MPL or antigen) are added to the emulsion in simple admixture.

Vaccines thus produced may be used to boost pre-existing anti allergy-peptide immunity of humans or animals.

Example 2 *Booster vaccinations of mice with IgE stanworth decapeptide constructs*

Four groups of 10 female (B6 x BALB/c)F1 mice were primed and boosted according to the following protocol. The stanworth decapeptide, KTKGSGFFVF, is described in EP 0 477 231 B1, and is an IgE peptide which is capable of inducing anti-IgE antibodies which inhibit histamine release from mast cells. The PS6B conjugates and oil in water emulsion adjuvants were produced as described in example 1, protein D-decapeptide conjugates were produced using standard commercially available peptide conjugation reagents following the manufacturers instructions (N-[γ-maleimidobutyryloxy] succinimide ester - GMBS). Amounts of antigen were adjusted to inject the same amount of IgE decapeptide per vaccination dose.

Peptide sequences were modified depending on carrier conjugation:

PD-deca: CKTKGSGFFVF

PS-deca: KTKGSGFFVF

All mice were primed on two occasions with a protein D-stanworth decapeptide conjugate intramuscularly, with 10 µg PD-deca conjugate on 25 µg of alum. All mice were boosted 14 months after the second priming dose in the following groups:

Table 2, Vaccine formulation

Groups and formulation of boosting doses	
A	Decapeptide conjugate with Protein D formulated in oil in water emulsion adjuvant with 3D-MPL and QS21
B	Decapeptide PS6B polysaccharide conjugate formulated in oil in water emulsion adjuvant with 3D-MPL and QS21

Bleedings were made at day 14 post II (after 2 PD-deca immunisations), at the day for boost with constructs to be evaluated (pre-boost) and at day 12 post III (post-boost) and anti-decapeptide and anti-carrier Ab titres measured in ELISA.

ELISA assay to determine anti-antigen antibody responses

The anti-peptide and anti-carrier immune responses were investigated using an ELISA technique outlined below.

Microtiterplates (Nunc) are coated with the specific antigen in PBS (4° overnight) with either:

Streptavidin at 2µg/ml (followed by incubation with biotinylated decapeptide (1µM) for 1 hour at 37°C), or P15-peptide, or Human IgE, or Protein D, or Polysaccharide 6B.

Plates are washed three times with PBS-Tween 20 0.1% before saturation with PBS-BSA 1%-Tween 20 0.1% (Sat buffer) for 1 hr at 37°. The 1° antibody = sera is added in two-step dilutions (in Sat buffer), and incubated 1 hr 30 minutes at 37°C. Plates are washed three times before incubation 1 hr at 37°C with an anti-mouse IgG coupled to HRP (Jackson). Plates are washed five times and plates are revealed with TMB

(BioRad) for 10 minutes at room temperature in the dark. The reaction is blocked with 0.4N H_2SO_4. Antibody responses are calculated as midpoint titers.

Results

The results of the boosting doses in terms of anti-decapeptide or carrier IgG responses are shown in the following tables (tables 3 to 5).

Table 3, Anti-Decapeptide titres

	Time	Anti Decapeptide titres (Individual mice)									
		1	2	3	4	5	6	7	8	9	10
A	Post II	43798	15641	19745	54054	20574	15604	25104	18268	3367	11108
	Pre III	464	1609	771	927	4332	3489	4298	232	3854	2399
	Post III	3028	16480	19824	12974	17518	47048	55296	18326	35583	19969
B	Post II	48446	36260	120833	74031	18132	6594	19575	63055	106326	49025
	Pre III	1964	1007	5159	331	1277	23	1355	8566	441	714
	Post III	8551	18811	22218	26601	7665	15534	12769	32984	18500	7965

Table 4, Anti-Protein D titres

	Time	Anti Protein D titres (Individual mice)									
		1	2	3	4	5	6	7	8	9	10
A	Post II	25141	17152	15506	22138	5784	22184	27337	11041	3449	3019
	Pre III	261	963	1102	802	1770	2516	2927	1576	1780	1963
	Post III	14758	14413	41567	18816	28377	31490	44510	43479	21535	44247
B	Post II	21675	19258	38065	52762	24642	12665	16093	52627	34975	21567
	Pre III	3080	1115	1231	367	1388	<100	953	2480	235	1550
	Post III	2514	754	641	239	908	<100	1083	1457	133	797

Table 5, Anti-PS6B titres

	Time	Anti PS6B titres (Individual mice)									
		1	2	3	4	5	6	7	8	9	10
B	Pre III	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	Post III	35	13	<10	<10	<10	<10	176	10	24	19

Discussion

The results above show that Deca-peptide-PS does give a boost when formulated with an adjuvant in the absence of peptide T-cell helper epitopes. In the deca-PS6B group, 9/10 mice have 4-fold increase in anti-deca titre after boosting in comparison to the pre-boost titres (4/10 have a 16-fold increase); whereas 10/10 of mice in group A (deca-protein D conjugate) have at least a 4-fold increase (only 2/10 show a 16-fold increase).

Claims

1. A composition comprising a plurality of allergy peptides linked by an inert carrier; characterised in that said allergy peptides are derived from IgE or IgE receptor, and that the inert carrier does not contain a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation.

2. A composition according to claim 1, characterised in that said inert carrier is selected from the group comprising: linear or branched polysaccharide, lipo-polysaccharides, poly-aminoacid homopolymers and block copolymers as well as blockcopolymers of aminoacids and polymers such as polyethyleneglycol or polycaprolactone, liposomes and other vesicular structures such as niosomes, novasomes, and ISCOMs and also particulate structures such as nanoparticles, supramolecular biovectors or microspheres composed of polymers.

3. A composition according to claim 1 or 2, wherein said inert carrier is the polysaccharide PS6B derived from *Streptococcus pneumoniae*.

4. A composition according to any one of claims 1 to 3, characterised in that said allergy peptide is a peptide derived from IgE.

5. A composition according to claim 4, wherein said peptide derived from IgE is selected from the group of peptides:

EDGQVMDVD
STTQEGEL
SQKHWLSDRT
GHTFEDSTKKCADSNPRGV
CADSNPRGV
CLEDGQVMDVDLL

CSTTQEGELA
CSQKHWLSDRT
RASGKPVNHSTRKEEKQRNGTL
GTRDWIEGE
PHLPRALMRSTTKTSGPRA
PEWPGSRDKRT
EQKDE
CRASGKPVNHSTRKEEKQRNGLL
CGTRDWIEGLL
CHPHLPRALMLL
APEWPGSRDKRTC
KTKGSGFFVF

6. A vaccine composition comprising a composition as claimed in any one of claims 1 to 5, and an adjuvant.

7. Use of an allergy vaccine comprising a plurality of allergy peptides linked by an inert carrier, characterised in that said allergy peptides are derived from IgE or IgE receptor, and that said inert carrier does not contain a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation; to boost an anti-allergy immune response in an individual susceptible to an allergic response, characterised in that the immune system of said individual has previously been primed with a composition comprising the allergy peptide and a carrier comprising a T-helper epitope.

8. A method of boosting an anti-allergy immune response, by administering a composition of claim 6 to an individual susceptible to an allergic response, characterised in that the immune system of said individual has previously been primed with a composition comprising the allergy peptide and a carrier comprising a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation.

9. A method of boosting an anti-allergy immune response as claimed in claim 8, characterised in that said carrier comprising a T-helper epitope is selected from the group comprising: protein D from Haemophilus Influenzae or fragment or derivative thereof, Tetanus toxin or fragment or derivative thereof, Diptheria toxin or fragment or derivative thereof, serum albumins, such as BSA, PPD, and KLH.

10. A composition as described in any one of claims 1 to 6 for use as a medicament.

11. A method of producing an allergy vaccine comprising manufacturing a composition as described in claims 1 to 6 and formulating said composition with an adjuvant.

12. A method of inducing and maintaining an anti-allergy effective immune response comprising: (a) administering to an individual a composition comprising an allergy peptide conjugated to a T-cell epitope containing carrier; and (b) a subsequent administration to said individual of a composition comprising said allergy peptide in the absence of a peptidic T-cell epitope containing carrier.

13. Use of an allergy peptide linked to an inert carrier; characterised in that said allergy peptide is derived from IgE or IgE receptor, and that said inert carrier does not contain a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation, in the manufacture of a medicament for the prophylaxis or treatment of allergy.

SEQUENCE LISTING

<110> SmithKline Beecam Biologicals s.a.

<120> Vaccine

<130> B45184

<160> 18

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IgE peptides

<400> 1
Glu Asp Gly Gln Val Met Asp Val Asp
1 5

<210> 2
<211> 8
<212> PRT
<213> Artificial Sequence

<400> 2
Ser Thr Thr Gln Glu Gly Glu Leu
1 5

<210> 3
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 3
Ser Gln Lys His Trp Leu Ser Asp Arg Thr
1 5 10

<210> 4
<211> 19
<212> PRT
<213> Artificial Sequence

<400> 4
Gly His Thr Phe Glu Asp Ser Thr Lys Lys Cys Ala Asp Ser Asn Pro
1 5 10 15
Arg Gly Val

<210> 5
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 5
Cys Ala Asp Ser Asn Pro Arg Gly Val
1 5

<210> 6
<211> 13
<212> PRT
<213> Artificial Sequence

<400> 6
Cys Leu Glu Asp Gly Gln Val Met Asp Val Asp Leu Leu
1 5 10

<210> 7
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 7
Cys Ser Thr Thr Gln Glu Gly Glu Leu Ala
1 5 10

<210> 8
<211> 11
<212> PRT
<213> Artificial Sequence

<400> 8
Cys Ser Gln Lys His Trp Leu Ser Asp Arg Thr
1 5 10

<210> 9
<211> 22
<212> PRT
<213> Artificial Sequence

<400> 9
Arg Ala Ser Gly Lys Pro Val Asn His Ser Thr Arg Lys Glu Glu Lys
1 5 10 15
Gln Arg Asn Gly Thr Leu
20

<210> 10
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 10
Gly Thr Arg Asp Trp Ile Glu Gly Glu
1 5

<210> 11
<211> 19
<212> PRT
<213> Artificial Sequence

<400> 11
Pro His Leu Pro Arg Ala Leu Met Arg Ser Thr Thr Lys Thr Ser Gly
1 5 10 15
Pro Arg Ala

<210> 12
<211> 11
<212> PRT
<213> Artificial Sequence

<400> 12

Pro Glu Trp Pro Gly Ser Arg Asp Lys Arg Thr
1 5 10

<210> 13
<211> 5
<212> PRT
<213> Artificial Sequence

<400> 13
Glu Gln Lys Asp Glu
1 5

<210> 14
<211> 23
<212> PRT
<213> Artificial Sequence

<400> 14
Cys Arg Ala Ser Gly Lys Pro Val Asn His Ser Thr Arg Lys Glu Glu
1 5 10 15
Lys Gln Arg Asn Gly Leu Leu
20

<210> 15
<211> 11
<212> PRT
<213> Artificial Sequence

<400> 15
Cys Gly Thr Arg Asp Trp Ile Glu Gly Leu Leu
1 5 10

<210> 16
<211> 12
<212> PRT
<213> Artificial Sequence

<400> 16
Cys His Pro His Leu Pro Arg Ala Leu Met Leu Leu
1 5 10

<210> 17
<211> 13
<212> PRT
<213> Artificial Sequence

<400> 17
Ala Pro Glu Trp Pro Gly Ser Arg Asp Lys Arg Thr Cys
1 5 10

<210> 18
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 18
Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
1 5 10

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/74716 A3

(51) International Patent Classification[7]: A61K 39/385, A61P 37/08

(21) International Application Number: PCT/EP00/05164

(22) International Filing Date: 6 June 2000 (06.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9913327.4 8 June 1999 (08.06.1999) GB

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: PRIEELS, Jean-Paul [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).

(74) Agent: DALTON, Marcus, Jonathan, William; SmithKline Beecham, Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
19 July 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/74716 A3

(54) Title: IGE PEPTIDES FOR ALLERGY IMMUNOTHERAPY

(57) Abstract: The present invention provides a composition for the prophylaxis or treatment of allergy. The composition comprises a plurality of allergy peptides linked together by an inert carrier; characterised in that said allergy peptides are derived from IgE or IgE receptor, and that the inert carrier does not contain a peptidic T-cell helper epitope which is capable of binding to an MHC molecule and is capable of stimulating T-cell proliferation, and in a preferred embodiment the carrier does not provide bystander T-cell cytokine help in the induction of the anti-peptide immune response. Also provided are methods of their manufacture and their use in medicine. There is also provided a method of treatment of allergy comprising priming the immune system with an IgE peptide/protein conjugate, followed by boosting the immune response with a composition of the present invention.

INTERNATIONAL SEARCH REPORT

:rnational Application No
PCT/EP 00/05164

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/385 A61P37/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 26365 A (UNITED BIOMEDICAL, INC.) 5 October 1995 (1995-10-05) cited in the application page 12, line 1 -page 13, line 21; claims	1-13
A	D.R. STANWORTH ET AL.: "Allergy treatment with a peptide vaccine." THE LANCER, vol. 336, 24 November 1990 (1990-11-24), pages 1279-1281, XP002157626 LONDON, GB the whole document	1-13
A	US 4 261 973 A (W.E. LEE ET AL.) 14 April 1981 (1981-04-14) claims	1-13

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 January 2001	05/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ryckebosch, A

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/05164

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 50460 A (SMITHKLINE BEECHAM BIOLOGICALS ET AL.) 31 August 2000 (2000-08-31) page 15, line 18 - line 28 page 17, line 8 - line 23 page 23, table 1	1,2,4,6, 10-13

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/05164

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9526365 A	05-10-1995	AU	2195395 A	17-10-1995
		CA	2186595 A	05-10-1995
		CN	1146772 A	02-04-1997
		EP	0811016 A	10-12-1997
		JP	9510975 T	04-11-1997
US 4261973 A	14-04-1981	GB	1578348 A	05-11-1980
		AT	359637 B	25-11-1980
		AT	592477 A	15-04-1980
		AU	520366 B	28-01-1982
		AU	2794277 A	22-02-1979
		BE	857869 A	17-02-1978
		CA	1091153 A	09-12-1980
		CH	641681 A	15-03-1984
		DE	2736223 A	23-02-1978
		DK	364277 A,B,	18-02-1978
		FR	2362156 A	17-03-1978
		JP	1016812 B	27-03-1989
		JP	1537301 C	21-12-1989
		JP	53024033 A	06-03-1978
		NL	7709025 A	21-02-1978
		NO	772848 A,B	20-02-1977
		SE	448147 B	26-01-1987
		SE	7709233 A	18-02-1978
WO 0050460 A	31-08-2000	AU	2672700 A	14-09-2000
		AU	3158900 A	14-09-2000
		WO	0050077 A	31-08-2000